REMARKS

Claims 1, 3-19, 26-27, and 34, of which claims 1, 3-13 and 15-19 are currently amended and claims 26-27 are withdrawn, are pending and appear in this application for the Examiner's review and consideration. Claims 2 and 28-33 are cancelled herein. The claims are amended to emphasize features of the present invention that are different from the cited prior art. The amendments to the claims are supported by the original specification and claims (*see, e.g.*, paragraph [0011] and [0013] of the published application). The specification is amended to include a specific reference to the prior applications. As no new matter is introduced, Applicants respectfully request that the claim and specification amendments be entered into the application at this time.

Priority

In response to the Examiner's comment on the priority claim, the specification is amended to include a specific reference to the prior applications, International Application No. PCT/IL02/00369, filed May 14, 2002, and U.S. Provisional Application No. 60/328,798, filed October 15, 2001. Therefore, the application now includes a proper claim of priority.

Claim Rejections -- 35 U.S.C. § 101

Claims 1, 7 and 8 are rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter. In response, claims 1, 7 and 8 are amended to recite that the insulin-producing cells are derived from human embryonic stem cell line. Claims 3-6, 9, 11-13, and 15-17 are also amended in the same manner. Support for this amendment can be found throughout the specification, for example at paragraphs [0011] and [0056] of the published application. Accordingly, the rejection based on § 101 should be withdrawn.

Claim Rejections -- 35 U.S.C. § 112

Claim 17 is rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement for the reasons set forth on pages 5-7 of the Office Action. Applicants respectfully traverse.

Applicants first note that the cells used in Halvorsen are adult beta-cells that were already fully differentiated at the time of transfection with hTERT, telomerase reverse transcriptase subunit (the catalytic telomerase subunit). Thus, the growth arrest observed in Halvorsen would have resulted from the adoption of a telomere-independent senescence

program by the fully differentiated cells such that senescence could no longer be circumvented by hTERT ectopic expression. Halvorsen is not a relevant reference because the present invention, in contrast to Halvorsen, relates to expression of hTERT in *pluripotent* human embryonic stem cells (hESCs), *prior to* their directed differentiation into insulin-producing cells (*see, e.g.,* published application, [0096]-[0123]).

Furthermore, Applicants would like to bring to the Examiner's attention the disclosures of Zalzman *et al.* (*Proc. Natl. Acad. Sci.*, 100(12):7253-58 (June 10, 2003)) (copy attached hereto as Exhibit A), which demonstrates that directing human fetal liver cells along the pancreatic beta-cell lineage under Pdx1 (pancreatic duodenal homebox transcription factor) regulation, and *after* retroviral introduction of hTERT, results in *numerous* Pdx1-activated beta-cell genes which produce, store and release insulin in response to glucose. Zalzman also notes that human fetal liver cells contain epithelial progenitor cells.

Present Applicants Skorecki and Tzukerman have also shown that overexpression of telomerase gene in hESCs does not adversely influence the differentiation capacity of these cells and, furthermore, that hESCs overexpressing the telomerase promoter are capable of proliferation while maintaining active telomerase promoter (*see* International Publication No. WO 03/066839).

Wege *et al.* (*Cell Transplantation*, 12(8):897-906 (2003)), a copy of which is attached hereto as Exhibit B, also shows that retroviral-mediated transduction of (hTERT), in proliferating human hepatocytes (PHH), results in maintenance of elongated telomeres and continued proliferation, while untransduced PHH progressively loses telomeric repeats and arrests after 30-35 cell divisions with telomeres of less than 5 kilo bases.

Therefore, the art does not teach that it is unpredictable whether hESCs that overexpress hTERT can be used to derive insulin-producing cells with decreased senescence, but supports and is instead consistent with the language of present claim 17.

With respect to the Examiner's statement that "[t]he specification does not provide any information on whether the hTERT coding sequence is stably expressed or integrated into a human ESC" (Office Action, p. 6), Applicants note that hESCs stably expressing hTERT were already known and published at the time this application was filed. A fully enabling disclosure was provided, for instance, in International Publication No. WO 03/066839, published August 14, 2003, as to stable transfection of hESC clones with hTERT promoter, construct of hTERT promoter-luciferase reporter used for the transfection, and hESC clones stably transfected with the hTERT promoter.

Accordingly, since the art does not teach unpredictability and there was relevant knowledge in the art at the time of the invention, Applicants respectfully submit that the present application provides sufficient guidance for one skilled in the art to practice the invention, and request that the § 112 rejection of claim 17 be withdrawn.

Claims 28-33 are also rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the enablement requirement. These claims, however, are cancelled without prejudice to Applicants' rights to file a divisional application for the subject matter of the claims, and this rejection is therefore moot.

Claim Rejections -- 35 U.S.C. § 102

Claims 1, 2, 5-6, 8, 12-16, 28, 30-31, and 33-34 are rejected under 25 U.S.C. § 102(a) as being anticipated by Lumelsky *et al.* (*Science*, 292:1389-94 (April 26, 2001)) for the reasons set forth on pages 8-9 of the Office Action. Applicants respectfully traverse.

Lumelsky merely teaches generation of cells that are derived from *mouse* ESCs and express insulin. These cells are *not isolated*, but, because "the majority of insulin-positive cells were localized in tight clusters in close association with neurons" (*see* p. 1390, lines 6-9), the cells generated in Lumelsky are contaminated with other cells, in particular neurons.

By contrast, the present claims are directed to *isolated* cell populations comprising insulin-producing cells derived from *human* ESCs. As is well known, human ESCs are fundamentally different from mouse ESCs. For example, the conditions for deriving and differentiating human ESCs are completely different from those required for deriving and differentiating mouse ESCs. The difficulty of directing human ESCs towards hematopoietic cells with techniques that are typically used for murine ESCs is explained in International Publication No. WO 01/34776, which provides: "There have also been some attempts to direct murine embryonic cell populations towards hematopoietic cells. . . . However, applying these teachings to primates has proven difficult. For example, in F. Li *et al.*, 92 Blood 368a (1998) there was a discussion of techniques for differentiation of rhesus embryonic stem cell lines using a stromal cell line and exogenous cytokines. However, that group has more recently reported that their techniques had inadequate formation of colonies." (p. 2, lines 8-22).

In addition, there are critical differences in the development of specific lineages and timing of embryonic genome expression between the primate and murine species. International Publication No. WO 96/22362, for example, explains such differences between human ESCs and mouse ESCs and the resulting differences in their applications as follows:

> Because mouse ES cells have the potential to differentiate into any cell type in the body, mouse ES cells allow the *in vitro* study of the mechanisms controlling the differentiation of specific cells or tissues. Although the study of mouse ES cells provides clues to understanding the differentiation of general mammalian tissues, *dramatic differences in primate and mouse development of specific lineages limits the usefulness of mouse ES cells as a model of human development*. Mouse and primate embryos differ meaningfully in the timing of expression of the embryonic genome, in the formation of an egg cylinder versus an embryonic disc . . . , in the proposed derivation of some early lineages . . . , and in the structure and function in the extraembryonic membranes and placenta . . . . Other tissues differ in growth factor requirements for development (e.g. the hematopoietic system . . . , and in adult structure and function (e.g. the central nervous system).

(p. 3, lines 2-27) (emphasis added).

Given such fundamental difference between human and murine ESCs, Lumelsky, which only teaches generation of unisolated cells derived from murine ESCs, cannot anticipate the present claims directed to human ESCs. In addition, in the interest of expediting the prosecution of this application, claims 1, 3-13 and 15-19 have been amended to recite that the present cell population is *isolated*, in contrast to the unisolated cells of Lumelsky. Support for this amendment is found throughout the specification, for example in paragraphs [0013] and [0132] of the published application.

Therefore, Applicants respectfully request that the rejections based on Lumelsky be withdrawn.

Claims 1, 7-8, 14, 28, 31, and 34 are rejected under 35 U.S.C. § 102(b) as being anticipated by Assady *et al.* (33rd Annual Meeting of the American Society of Nephrology, 2000 Renal Week, Canada, Program and Abstract, vol. 11 (September 2000)) for the reasons stated on page 9 of the Office Action. Applicants respectfully traverse.

Applicants first note that Assady, as explained in its abstract, is directed to a model system for *spontaneous* differentiation of pluripotent undifferentiated hESCs into lineage specific stem cells that include an *unspecified incidence* of insulin-producing cells, and therefore does not teach, disclose or suggest the isolated cell populations as recited in the present claims.

Moreover, in contrast to Assady, which does not provide any disclosure or suggestion with respect to regulation, enrichment, selection, isolation or cloning of an insulin-producing

cell population derived from hESCs, the present invention enables directed differentiation of hESCs to insulin-producing cells and thus enables directed enrichment of hESCs for insulin-producing cells. Assady also does not disclose or suggest specific genetic modifications of hESCs, including specific gene sequences encoding, for example, an insulin promoter or telomerase for promoting the formation of a cell population comprising a pluripotent precursor of insulin-producing cells as disclosed in the present invention.

Further, in the interest of expediting prosecution of this application, claims 1 and 7 have been amended to recite that the claimed cell population is enriched for insulin-producing cells derived from human ESC line. Such claim recitation emphasizes the difference between the claimed cell population and the disclosures of Assady.

Accordingly, the § 102 rejections based on Assady should be withdrawn.

Likewise, the rejection of claims 1, 2, 5, 7-9, 12, and 15 based on Peakman *et al.* (*Transplantation*, 57:384-393 (1994)) should also be withdrawn. Peakman discloses isolation of beta-cells from human pancreas using proteolytic enzymes. The reference states that the tested enzymes produce a low yield of beta-cells and/or damage the isolated cells because of over-digestion, among other reasons, and discloses that the beta-cell preparations obtained by digestion of human pancreas by proteolytic enzymes may contain variable amounts of proteolytic enzyme impurities (*see* p. 391). Peakman also discloses that the problem with the islet grafts is that they are *short-lived* (p. 389, bottom of the right column).

The present cell population differs from the disclosures of Peakman in at least several respects. First, the present cell population, which is derived from human ESC line, is *long-lived*, unlike the islet grafts of Peakman (*see* [0015] of the published application). Second, because obtaining the cell population does not involve application of proteolytic enzymes according to the present application, the present cell population is substantially devoid of enzymatic impurities or activities associated with the presence of proteolytic enzymes. Third, the present cell population comprises beta-cell precursors capable of maintaining their pluripotency (*see* [0017]-[0018]) because they are derived from pluripotent hESC. In this respect, Applicants emphasize that, as recited in every claim of this application, the claimed population of insulin-producing cells is derived from hESC line.

Accordingly, Applicants respectfully request that the rejections based on Peakman be withdrawn.

All the rejections under § 102 should therefore be withdrawn.

Claim Rejections -- 35 U.S.C. § 103

Claims 1-16, 18-19, 28, 30-31, and 33-34 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Soria *et al.* (*Diabetes*, 49:1-6 (2000)) and in view of Chrenek *et al.* (*Theriogenology*, 50:659-666 (1998)) for the reasons set forth on pages 10-12 of the Office Action. Applicants respectfully traverse.

Soria is directed to production of insulin-secreting cells derived from *mouse* embryonic stem cells. By contrast, the present claims are directed to insulin-secreting cells derived from *human* ESCs. As explained with respect to Lumelsky, human ESCs are fundamentally different from murine ESCs, especially in their conditions for derivation and differentiation, in their development of specific lineages, and in the timing of embryonic genome expression, and therefore present utility for different applications.

Accordingly, the disclosures relating to murine ESCs as provided in Soria cannot render obvious the preparation, differentiation, lineage commitment, *in-vivo* and *in-vitro* effects, among others, of human ESCs.

Chrenek, which merely teaches the use of a serum-free medium supplemented with several growth factors, including insulin, transferrin and sodium selenite, for the development of *rabbit zygotes*, fails to address the deficiencies of Soria. Not only is there no motivation in Soria for using the particular growth media that would be suitable for culturing human ESCs, but such deficiency is not rectified by Chrenek, whose culturing medium is taught as a medium for growing rabbit zygotes, which are essentially different from both human ESCs of the present invention and mouse ESCs of Soria. Thus, one skilled in the art would not have any motivation to apply the disclosures of Soria and Chrenek to human ESCs, nor would there be a reasonable expectation of success in deriving insulin-producing cells from hESCs or in culturing hESCs by supplementing the media with the growth factors taught in Chrenek. In this respect, the Examiner's statement that "the substitution of human ESCs for mouse ESCs as the source of cells for derivation of insulin producing cells comprises a minor modification of the teachings of Soria et al." (Office Action, p. 12) is incorrect, because the differences between human and mouse ESCs are so significant that substituting one for the other cannot be considered a minor modification.

Accordingly, at least because Soria neither teaches, discloses, nor suggests the preparation, differentiation, lineage commitment, and *in-vivo* and *in-vitro* effects that relate to human ESCs, and because Chrenek fails to address the deficiencies of Soria, Applicants respectfully submit that the § 103 rejections are inappropriate and should be withdrawn.

In view of the above, it is believed that the entire application is in condition for allowance, early notification of which would be appreciated. Should the Examiner not agree with this position, a telephone or personal interview is requested to resolve any remaining issues and expedite allowance of this application. Please call the undersigned to expedite the allowance of all claims in this application.

Respectfully submitted,

Date: 4/22/05

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